

	Hits	Search Text	DBs
1	1	("6455279").PN.	USPAT
2	0	protein with prokaryot\$2 with (construct or vector) with OmpA	USPAT
3	3	protein with prokaryot\$2 with (construct or vector) with OmpA	USPAT; US-PGPUB; EPO; JPO; DERWENT
4	4	prokaryot\$2 with (construct or vector) with OmpA	USPAT; US-PGPUB; EPO; JPO; DERWENT

	Hits	S arch Text	DBs
1	1	("6455279").PN.	USPAT
2	0	protein with prokaryot\$2 with (construct or vector) with OmpA	USPAT
3	3	protein with prokaryot\$2 with (construct or vector) with OmpA	USPAT; US-PGPUB; EPO; JPO; DERWENT
4	4	prokaryot\$2 with (construct or vector) with OmpA	USPAT; US-PGPUB; EPO; JPO; DERWENT
5	3	protein with prokaryot\$2 with (construct or vector) with Omp?	USPAT; US-PGPUB; EPO; JPO; DERWENT
6	4	prokaryot\$2 with (construct or vector) with Omp?	USPAT; US-PGPUB; EPO; JPO; DERWENT
7	4	prokaryot\$2 NEAR20(construct or vector) NEAR20 Omp?	USPAT; US-PGPUB; EPO; JPO; DERWENT

	Time Stamp
1	2003/07/10 14:49
2	2003/07/10 14:50
3	2003/07/11 11:27
4	2003/07/11 11:28
5	2003/07/11 11:28
6	2003/07/11 11:28
7	2003/07/11 11:28

ACCESSION NUMBER: 1998191875 MEDLINE
DOCUMENT NUMBER: 98191875 PubMed ID: 9518455
TITLE: Recombinant production of cyanovirin-N, a potent human immunodeficiency virus-inactivating protein derived from a cultured cyanobacterium.
AUTHOR: Mori T; Gustafson K R; Pannell L K; Shoemaker R H; Wu L; McMahon J B; Boyd M R
CORPORATE SOURCE: Laboratory of Drug Discovery Research and Development, Division of Cancer Treatment, Diagnosis and Centers, National Cancer Institute-FCRDC, Frederick, Maryland 21702-1201, USA.
SOURCE: PROTEIN EXPRESSION AND PURIFICATION, (1998 Mar) 12 (2) 151-8.
Journal code: 9101496. ISSN: 1046-5928.
PUB. COUNTRY: United States
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; AIDS
ENTRY MONTH: 199805
ENTRY DATE: Entered STN: 19980609
Last Updated on STN: 19980609
Entered Medline: 19980526

AB Here we describe the recombinant production and purification of a novel anti-human immunodeficiency virus (HIV) protein, cyanovirin-N (CV-N), in *Escherichia coli*. Initial attempts to **express** CV-N using a **vector** containing an **ompA** signal peptide sequence resulted in production of an intractable mixture of the full-length (101 amino acid residue) protein and a truncated form lacking the first two N-terminal amino acids. The truncated protein was observed regardless of the host cell line, culture conditions, or induction time. These observations suggested that an as yet unidentified protease or peptidase was responsible for proteolytic cleavage between the second and third N-terminal amino acids of CV-N when presented as an **ompA**-CV-N fusion protein. When the **ompA** signal peptide sequence was replaced by a **pelB** signal peptide sequence, CV-N was produced in high yield as a single, homogeneous protein. This was confirmed by electrospray ionization mass spectrometry and N-terminal sequencing. This expression system provides a basis for large-scale production of clinical grade CV-N for further research and development as an anti-HIV microbicide.

ACCESSION NUMBER: 1995:119117 BIOSIS

DOCUMENT NUMBER: PREV199598133417

TITLE: Improved crystals of the toxic protein MAP by protein engineering towards the host specificity.

AUTHOR(S): Ago, Hideo (1); Habuka, Noriyuki (1); Kataoka, Jiro (1); Furuno, Masahiro (1); Tsuge, Hideaki (1); Noma, Masana (1); Miyano, Masashi (1); Wang, Bi-Cheng; Xuong, Nguen Huu

CORPORATE SOURCE: (1) Life Sci. Res. Lab., Japan Tobacco Inc., 6-2 Umegaoka, Midori-ku, Yokohama 227 Japan

SOURCE: Acta Crystallographica Section D Biological Crystallography, (1994) Vol. 50, No. 4, pp. 404-407. ISSN: 0907-4449.

DOCUMENT TYPE: Article

LANGUAGE: English

AB Mirabilis anti-viral protein (MAP) is a ribosome-inactivating protein from *Mirabilis jalapa* L. Since MAP is effective over a broad spectrum of species, the protein is difficult to **express** in heterologous hosts such as *Escherichia coli*. Recently, we obtained a MAP mutant, Y72F which exhibits a lower (1/100) activity against *E. coli* ribosomes while retaining almost full activity against mammalian cells (Habuka, Miyano, Kataoka, Tsuge & Noma (1992). *J. Biol. Chem.* 267, 7758-7760). For the crystallographic studies, the Y72F MAP expression **vector** with an **OmpA** leading sequence was constructed and expressed in *E. coli*. The Y72F MAP mutant was then isolated and purified from the cell culture medium. Crystals were grown using the crystallization conditions for the native MAP crystals (Miyano et al. (1992). *J. Mol. Biol.* 226, 281-283): 50% ammonium sulfate containing 50 mM ammonium citrate and 2 mM adenine sulfate, pH 5.4. The crystals belong to space group P3-121 (or P3-221) with $a = b = 104.1$ and $c = 134.3$ Å. The crystals are isomorphous with the wild-type crystals but diffract to higher resolution. Imaging-plate photographs of the Y72F mutant showed sharp intense spot

L2 ANSWER 10 OF 17 MEDLINE DUPLICATE 4

ACCESSION NUMBER: 94071826 MEDLINE
DOCUMENT NUMBER: 94071826 PubMed ID: 8250844
TITLE: Expression of functional human retinol-binding protein in Escherichia coli using a secretion **vector**.
AUTHOR: Sivaprasadarao A; Findlay J B
CORPORATE SOURCE: Department of Biochemistry and Molecular Biology, University of Leeds, U.K.
SOURCE: BIOCHEMICAL JOURNAL, (1993 Nov 15) 296 (Pt 1) 209-15.
Journal code: 2984726R. ISSN: 0264-6021.
PUB. COUNTRY: ENGLAND: United Kingdom
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199312
ENTRY DATE: Entered STN: 19940201
Last Updated on STN: 19970203
Entered Medline: 19931229

AB In order to **express** human serum retinol-binding protein (sRBP) in Escherichia coli in a form that is structurally indistinguishable from the native protein, we placed the coding sequence of the RBP cDNA next to that of the outer membrane protein A (**OmpA**) signal sequence in the secretion **vector**, pIN-III-OmpA1. However, this construct did not generate detectable expression of RBP in E. coli. When the DNA fragment consisting of the ribosome-binding site and the **OmpA**-RBP fusion sequence was subcloned downstream to the T7 promoter of pKS-Bluescript, however, the resultant construct (pOmp-RBP2) gave low but detectable secretion of RBP into the periplasm. Deletion of the 3' untranslated region of the RBP cDNA (pOmp-RBP3) further improved the expression (by approx. 20-fold). After charging with retinol, the secreted RBP was purified from the periplasm on a transthyretin-affinity resin. The purified protein exhibited all the three molecular recognition properties characteristic of sRBP, i.e. it interacted with retinol, transthyretin and its cell-surface receptor. Comparison of the receptor binding properties of the recombinant RBP (rRBP) with those of the serum protein revealed that while the affinity of rRBP is similar to sRBP (50 +/- 20 nM), the Bmax of the rRBP is about 6-8-fold higher. This indicates that a major proportion of RBP, isolated from serum, is incapable of interacting with the receptor.

L2 ANSWER 12 OF 17 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 94108390 MEDLINE
DOCUMENT NUMBER: 94108390 PubMed ID: 1342596
TITLE: The N-terminal amino acid sequence is essential for
foot-and-mouth disease virus replicase activity.
AUTHOR: Pacheco A B; Brindeiro R M; Soares M A; de-Almeida D F;
Tanuri A
CORPORATE SOURCE: Instituto de Biofisica, Universidade Federal do Rio de
Janeiro, Brasil.
SOURCE: BRAZILIAN JOURNAL OF MEDICAL AND BIOLOGICAL RESEARCH,
(1992) 25 (7) 659-66.
Journal code: 8112917. ISSN: 0100-879X.
PUB. COUNTRY: Brazil
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199402
ENTRY DATE: Entered STN: 19940228
Last Updated on STN: 19980206
Entered Medline: 19940217

- AB 1. Foot-and-mouth disease virus replicase was expressed by fusing its
cDNA to the **OmpA** signal peptide coding sequence present in the
pIN-III **ompA** series **vectors**. 2. Two constructions
were developed to **express** either a full-length or truncated
enzyme lacking the 20 amino acids at the N-terminal end. Bacterial
extracts expressing the recombinant proteins were submitted to SDS-PAGE
and the presence of the replicase was revealed by immunoblotting. The
truncated form exhibited a higher mobility and the relative positions of
the proteins show that the signal peptide was removed. 3. The biological
activity of these two molecules was tested using a poly(A)-dependent
oligo(U)-primed poly(U)-polymerase assay. The full-length replicase is
active. The aminoterminal truncated enzyme had 0.02% activity of the
intact one. 4. This result indicates the importance of the twenty
N-terminal amino acids for the activity of FMDV RNA-dependent RNA
polymerase.

L2 ANSWER 16 OF 17

MEDLINE

DUPLICATE 6

ACCESSION NUMBER: 89153585 MEDLINE
DOCUMENT NUMBER: 89153585 PubMed ID: 2646152
TITLE: Expression of ricin B chain in Escherichia coli.
AUTHOR: Hussain K; Bowler C; Roberts L M; Lord J M
CORPORATE SOURCE: Department of Biological Sciences, University of Warwick,
Coventry, England.
SOURCE: FEBS LETTERS, (1989 Feb 27) 244 (2) 383-7.
Journal code: 0155157. ISSN: 0014-5793.
PUB. COUNTRY: Netherlands
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 198904
ENTRY DATE: Entered STN: 19900306
Last Updated on STN: 20021218
Entered Medline: 19890413

AB DNA encoding ricin B chain was fused to that encoding the E. coli
OmpA signal peptide using the expression secretion **vector**
pIN-111-**ompA**. When induced, E. coli cells transformed with the
recombinant plasmid **express** ricin B chain. The recombinant
product accumulates in the periplasmic space in a soluble, biologically
active form.